IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

HONOLD et al.

Serial Number: 09/203,500

Filed: December 1, 1998

Attny Docket No. 100564-08025

Group Art Unit: 1636

Examiner: W. Sandals

For: OPTIMIZATION OF CELLS FOR ENDOGENOUS GENE ACTIVATION

RESPONSE UNDER 37 CFR 1.121

Commissioner for Patents
Washington, D.C. 20231

November 17, 2000

Sir:

This is a Response to the Office Action dated July 17, 2000, a Petition for Extension of Time for one month from October 17, 2000 from November 17, 2000 attached hereto.

Claims 20-43 remain pending.

Applicants note that the Examiner has withdrawn the rejection of claims 20-28, 30-36 and 39 under 35 USC §102(e) as being anticipated by the '977 patent. In addition, the Examiner has withdrawn the rejection under 35 USC §112, second paragraph.

The Examiner maintains that claims 20-43 are unpatentable over the '977 patent in view of WO '012, WO '650 and Cruz et al.

On page 3 of the Office Action, the Examiner states his belief as to what is claimed in the present invention. The Examiner notes that the claims are drawn to a process of changing the expression of a nucleic acid sequence which is present endogenously in a eukaryotic cell by transfecting the cell with a vector which encodes a marker gene and a gene of interest operably linked to an expression control sequence flanked by recombinase sites, which is flanked by sequences for homologous recombination, and where the

encoded gene of interest is expressed. The Examiner notes that the gene of interest may be DHFR.

This statement of the invention is clearly incorrect. None of the several different embodiments of vectors used in the present invention contains a gene of interest operably linked to an expression control sequence. Rather, the "gene of interest" is **an endogenous gene which is already present in the eukaryotic cell**.

For example, in the embodiment claimed in claims 20 and 24, a vector is presented having a heterologous expression control sequence/amplification gene and a positive selection marker gene flanked by target sequences for a site-specific recombinase. This construct is flanked by DNA sequences which are homologous to the target genome to facilitate homologous recombination. There is absolutely no heterologous gene of interest operably linked to the expression control sequence in the vector of these claims. Rather, the entire idea behind the embodiments of claims 20 and 24 is to change the expression of a nucleic acid sequence which is present endogenously in the cell. See the preamble for claim 20. The Examiner clearly misunderstands the intention of the present invention if he believes that a heterologous gene of interest is present in any of the vectors used and/or claimed in claims 20 and 24.

As applicants previously noted, the vector of claim 25 contains a heterologous expression control sequence/amplification gene and a positive selection marker gene flanked by two target sequences for a site-specific recombinase. Again, there is no heterologous gene of interest operably linked to the expression control sequence in the vector.

Independent claims 28 and 32 involve the use of a vector having a nucleic acid sequence which binds an activator protein (e.g., HIF binder) and a positive selection marker gene flanked by DNA sequences which are homologous to the target genome. In this case, the nucleic acid sequence which binds an activator protein acts as the expression control sequence. Yet, as with the embodiments discussed above, there is no heterologous gene of interest operably linked to this activator protein binding nucleic acid sequence.

Claim 35 involves the use of a vector having a heterologous expression control sequence which is operably linked with a reporter gene and non-encoding nucleic acid sequences on the 5'-side and/or the 3'-side from the region of the target gene. Again, there is no heterologous gene of interest operably linked to this expression control sequence.

Claim 36 involves the use of a vector having target sequence for a site-specific recombinase which are flanked by DNA sequences which are homologous to a DHFR nucleic acid sequence which is present endogenously in the target cell. In this particular case, the DHFR nucleic acid sequence is present endogenously in the cell in order to allow a homologous recombination to integrate the target sequence for a site-specific recombinase. In this case, the DHFR "gene of interest" (as characterized by the Examiner) is not linked to a heterologous expression control sequence.

Claim 39 is directed to a vector having a DHFR-encoding sequence, a nucleic acid sequence to be amplified and (optionally) a positive selection marker gene, where this construct is flanked by two recombinase target sequences. In this case, the nucleic acid

sequence to be amplified is not operably linked to a heterologous expression control sequence.

Thus, applicants fail to see how any of the embodiments of the invention discussed above fit the characterization of the invention asserted by the Examiner.

Likewise, the Examiner is again mischaracterizing the cited references.

On pages 3-4 of the Office Action, the Examiner asserts that the '977 patent discloses a process of changing the expression of a nucleic acid sequence which is present endogenously in a eukaryotic cell. The Examiner broadly cites to columns 1, 2 and 4-5, but does not provide any specific location where this process is allegedly taught.

As applicants have previously noted in detail, the invention in the '977 patent is used to enhance integration of a construct of interest which contains a consensus sequence TTAAAA for insertion into a genome, with one or more sequences of interest joined to the consensus sequence. These sequences of interest, which may be coding or non-coding, preferably code for a peptide of interest, an antisense sequence, a regulatory sequence, or the like. See column 5, lines 14-39.

It is clear that the expression cassette used in the '977 patent contains, as an essential feature, a transcribed sequence of interest (i.e., a exogenous gene). There is absolutely no disclosure whatsoever regarding the activation of endogenous genes, as asserted by the Examiner.

While it is true that the '977 patent discloses, in a general manner, a combination of homologous recombination and the introduction of site-specific recombinase-recognition sites (see column 4, lines 17-26), an explicit combination of endogenous gene activation and site-specific recombination has not been disclosed.

A further difference between the '977 patent and the present invention is that the recombinase-recognition sequences according to the '977 patent are introduced in the form of tandem multicopy sequences (see column 7, lines 33-38). In this manner a further sequence (which is not specified in detail) can be inserted and removed afterwards, subsequent to successful homologous recombination . Thus, according to the '977 patent a two-step process can be employed.

In contrast thereto, the construct according to the invention contains two site-specific recombination sequences that are not located next to each other. Between both sequences an expression control sequence to be modified and the selection marker gene are situated. Thus, by means of the process according to the invention, a selection of successful homologous recombination (including a correct insertion of the new expression control sequence and the insertion of the recombinase recognition sequences) is performed in a single step. The process according to the prior art is much more cumbersome, since the homologous recombination has to be carried out in a first step, whereby the function of the target gene cannot yet be modified as desired. Only in a second step can the functional sequences (which are not precisely specified in the patent) be inserted with the help of the site-specific recombinase. This method causes much more work, since at least two different positive selection markers (i.e., one selection marker for

the homologous recombination and the second selection marker for the site-specific recombination) are necessary for controlling the success.

The one-step process according to the present invention is far more elegant and cannot be derived from the '977 patent. On the contrary, applicants submit that the two-step process of the '977 patent actually teaches away from the present invention.

The Examiner also mischaracterizes the teachings of WO '012. In page 4 of the Office Action, the Examiner asserts that WO '012 also teaches a process for changing the expression of a nucleic acid sequence which is present endogenously in a eukaryotic cell. Again, the Examiner does not point to any particular section for support; rather, the Examiner merely cites to the abstract, the summary, the claims, the figures and pages 12-22.

As applicants discussed in detail in the previous Response, WO '012 discloses a genetic construct comprising a recombinase genetic unit (e.g., a *cre* gene) under the control of a first promoter and a transgene unit under the control of a second promoter, wherein the recombinase genetic unit and the transgene unit are linked and flanked by two recombination loci. Optionally the genetic construct can further comprise left order and right order sequences to facilitate its *in vivo* insertion into chromosomal DNA. As applicants have previously noted, WO '012 neither includes a reference to endogenous gene activation nor to homologous recombination.

Applicants have previously noted that WO '650 relates to endogenous gene expression, however there is no disclosure to the use of site-specific recombinase recognition sequences and the use of the claimed constructs, which are flanked by

recombinase recognition sequences. One of ordinary skill in the art would not have considered combining WO '650 and WO '012, since these documents each relate to totally different technical fields (heterologous gene expression on the one hand and endogenous gene activation on the other hand).

The Cruz publication describes the inactivation of a DHFR gene in a protozoal cell. There is no reference whatsoever to site-specific recombinase and the recognition sequences thereof. Thus, Cruz adds nothing besides the specific teaching therein to the rejection.

The Examiner has repeated his response to applicants' arguments, as previously presented in the Advisory Action of April 10, 2000. The Examiner's statements are merely boiler plate, and do not specifically address any of applicants' specific arguments. This is clearly in violation of both the letter and spirit of MPEP §707.07(f). Applicants again request that the Examiner specifically address the arguments of record.

In summary, the Examiner has not made a proper prima facie case of obviousness. In addition, the Examiner has failed to provide substantive comments addressing applicants' detailed and specific arguments on the references of record. Applicants respectfully submit that, if this case is not now considered in condition for allowance, the Examiner should clearly state on the record his reasons for continuing to advance these unfounded objections.

In the event this paper is not timely filed, applicants hereby petition for an appropriate extension of time. The fee for this extension may be charged to our Deposit Account No. 01-2300, along with any other additional fees which may be required with respect to this paper.

Respectfully submitted,
Arent Fox Kintner Plotkin & Kahn

Richard J. Berman
Attorney for Applicant(s)
Registration No. 39,107

1050 Connecticut Avenue, NW, Suite 600
Washington, D.C. 20036-5339
(202) 638-5000

RJB:ccd